ANATOMICAL ASYMMETRY OF THE RETROCEREBRAL COMPLEX IN THE CRICKET, *Gryllus locorojo* (ORTHOPTERA: GRYLLIDAE)

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SUMMARY

In the adult crickets, *Gryllus locorojo*, the location of retrocerebral glands within the head capsule was found to be asymmetric. The *corpora cardiaca* ventral bridge is prominently biased to the right from the sagittal line of the head capsule as well as from the central position within the retrocerebral complex. The location of the whole retrocerebral complex has a tendency to be rightward lateralized within the head capsule. Possible anatomical lateralization of retrocerebral glands in other cricket species needs to be tested. The asymmetric location of *corpora cardiaca* and aorta, the structures most closely associated with each other, could be a new trait in *G. locorojo* and potentially other gryllids.

Keywords: Corpora cardiaca, corpora allata, retrocerebral complex, aorta, morphology.

RESUMEN

En los grillos adultos, *Gryllus locorojo*, se encontró que las glándulas retrocerebrales dentro de la cápsula de la cabeza están asimétricas. El puente de la *corpora cardiaca* ventral está prominentemente inclinado a la derecha de la línea sagital de la cápsula de la cabeza, como también en relación a la posición central dentro del complejo retrocerebral. La ubicación del complejo retrocerebral en conjunto tiene una tendencia a estar lateralizado hacia la derecha dentro de la cápsula de la cabeza. La posible lateralización anatómica de las glándulas retrocerebrales en otras especies de grillos requiere ser verificado. La ubicación asimétrica de la *corpora cardiaca* y de la aorta, las estructuras más estrechamente relacionadas entre sí, podrían ser un nuevo rasgo distintivo en *G. locorojo* y potencialmente otros gryllidos.

Palabras clave: Corpora cardiaca, corpora allata, complejo retrocerebral, aorta, morfología.

INTRODUCTION

The retrocerebral complex (RCC) is a part of insect neuroendocrine system involved in the control of most aspects of insect physiology. Typically, the RCC consists of paired *corpora cardiaca* (CC) and paired *corpora allata* (CA) (Cazal 1948). The CC consist of axonal endings of neurosecretory cells and their own intrinsic endocrine (glandular) cells and, thus, represent a combined structure functioning as both neurohemal organ and classical endocrine gland (Klowden 2007). Among neurohormones released by the CC are prothoracicotropic (activating the prothoracic glands to produce the ecdysteroid molting hormone), diuretic, and antidiuretic hormones (neuroparsins, ion transport peptide, and other hormones controlling insect salt and water homeostasis) (Kind *et al.* 1983, Klowden 2007, Meredith *et al.* 1996, Schooley *et al.* 2012, Smith & Rybczynski 2012). Glandular cells of the CC produce adipokinetic and hypertrehalosemic hormones (increasing the lipid and trehalose concentrations in the haemolymph) (Siegert 1999, Schooley *et al.* 2012). The CA are mainly composed of endocrine cells. The main function of the CA is the biosynthesis of juvenile hormone which modulates molt quality (simple or methamorphic) in the larvae and regulates vitellogenesis in the adult insects (Kind *et al.* 1983, Klowden 2007).

The CC are joined to the cerebrum via *nervi corporis cardiaci*, the CA are joined to the CC and/or to the suboesophageal ganglion via *nervi corporis allati*. The left and right CC may be fused in an unpaired structure, as well

as the left and right CA. The variations of this RCC anatomical scheme are characteristics of various insect taxa (Cazal 1948). In Saltatoria e.g., the CC are fused with each other in their caudal parts, the CA are separated from each other, and both allatal nerves joining each *corpus allatus* with the CC and with the suboesophageal ganglion are present (Cazal 1948, Gaude 1975). A ring gland in higher dipterans represents the strongest modification of RCC, where the CC and CA are assembled with the prothoracic glands in a single ring-shaped structure (Klowden 2007).

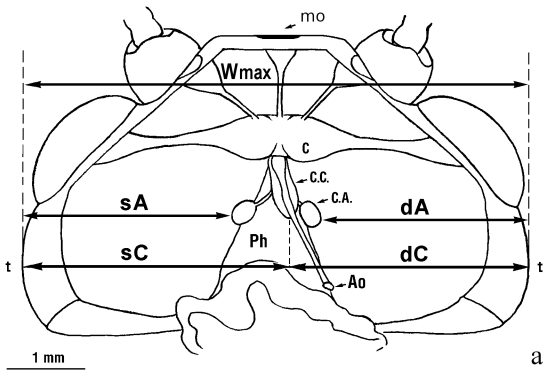
Typically, the RCC is located centrally in the insect body. While working with the cricket *Gryllus locorojo* Weissman and Gray 2012 (previously reported as "*Gryllus argentinus*"), the right side lateralization of the RCC location inside the head capsule was noticed. The aim of the present study was to describe and to statistically confirm this anatomical asymmetry. The confirmation of noticed asymmetry of RCC may be important for the further anatomical characterization of cricket species.

METHODS

Adult crickets of the species *Gryllus locorojo* were kindly donated by the Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences (the history and conditions of the year-round culture of these crickets are well described by Zhemchuzhnikov & Knyazev (2012)). Species identification was confirmed using Shestakov & Vedenina (2012) and Weissman *et al.* (2012).

The crickets were sacrificed by immersion in 70 % ethanol and preserved in the same medium for further use in dissections and asymmetry measurements. The cricket heads were prepared in a Petri dish filled with 70 % ethanol using tweezers and dissecting needles under a binocular microscope equipped with a scale and a reticle grid (MBS-9, LOMO, Russia). Heads were oriented dorsal-up for dissection. The dorsal head integument and tissue surrounding RCC were gently removed. To standardize the measurements of RCC location parameters, the heads of the crickets were oriented in a position in which the dorsal and ventral edges of the median ocellus (mo) are visibly merged into a single line (designated in Figure 1a). Fresh material prepared analogously in an aqueous solution of NaCl (0.75 % (w/v)) was used only for general description of RCC location, including the description of asymmetric topography without measurements. Drawings were performed using reticle grid and plotting paper.

The numbers of females and males used for description of the RCC location were 32. Among them, ten adult females were used for detailed measurements of the RCC topography parameters. The primary parameters (Figure 1) were measured using an ocular scale (the value for the smallest scale division was 25 μm at the magnification used). The parameters measured are: *sC* and *dC*, the distances from the left and right temples, respectively, to the projection of the caudal (ventral) CC bridge on the line connecting the temples; the sum of them is the length of the line connecting the temples or the maximal head width (*Wmax*); *sA* (*dA*), the distance from the left (right) temple to the projection of most lateral contour of nearest *corpus allatus* on the line connecting the temples; the result of subtraction of (*sA*+*dA*) from *Wmax* is the RCC width; *sR* (*dR*), the distances from the projection of the medial border of left (right) *corpus allatus* to the projection of the CC bridge caudal point on the line connecting the temples. On the basis of these primary parameters, the secondary (relative) parameters were calculated and expressed in percents of *Wmax*: *C/Wmax* (*s*, left; *d*, right), the *sC* and *dC*, related to *Wmax*; *A/Wmax* (*s*, left; *d*, right), the *sA* and *dA*, related to *Wmax*; *R/Wmax* (*s*, left; *d*, right), the *sR* and *dR*, related to *Wmax*.



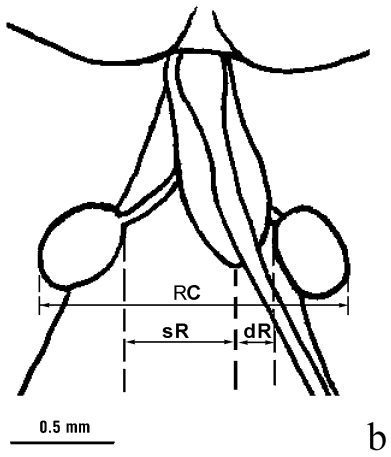


Figure 1. The adult cricket head (dorsal view; the dorsal epicranium is removed) with designated parameters used for the estimation of asymmetry of CC, CA, and whole RCC location in the head capsule (a) and of asymmetric location of CC within RCC (b) (the magnified RCC image from (a)). Designations of structures (here and in Figures 2 and 3): Ao, aorta; C, cerebrum; C.C., *corpus cardiacus* (single); C.A., *corpus allatus* (single); mo, median ocellus; Ph, pharynx; t, temple. Designations of topography parameters: sC and dC, the distances from the left and right temples, respectively, to the projection of the caudal (ventral) CC bridge on the line connecting the temples; sA (dA), the distance from the left (right) temple to the projection of most lateral contour of nearest *corpus allatus* on the line connecting the temples; sR (dR), the distances from the projection of the medial border of left (right) *corpus allatus* to the projection of the CC bridge caudal point on the line connecting the temples. Other designations: RC, the RCC width; Wmax, maximal head width.

Among the methods for studying asymmetry, the comparisons of sizes of left and right parts of anatomical structures as well as calculating the laterality indexes are common approaches to detect leftward and rightward lateralization (Toga & Thompson 2003). In the present study, a relevant approach to detect the direction of CC bridge and whole RCC biases was to compare pairwise the left and right distances measured. Only distribution-free descriptive and comparative statistical procedures were applied to the data collected. The Shapiro-Wilk test (the most powerful normality test according to Razali & Wah (2011)) was used to check the normality of underlying distributions prior to proceeding the description and comparisons. Since a departure from normality was detected (the null hypothesis that the underlying distribution is normal was rejected with the type I error less than 5 %), the parametric methods widely used for estimation asymmetry, such as two-way ANOVA, could not be applied (Glantz 1994; Kvam & Vidacovic 2007). The data were presented in the form of median values and quartiles (Me (q1; q3)), and the exact binomial test (the sign z-test) was used in order to test for the null hypothesis that the particular RCC part is equally likely to be biased to the right or to the left side. The applications of binomial tests can be found in Anfora *et al.* (2011) (in parallel with ANOVA), Arcadi & Wallauer (2011), Rutledge & Hunt (2004) with some specific features depending on the experimental design, the lateralization quality (structural or functional), and the level of lateralization (individual or populational). The binomial approach is used even for much more complicated cases of lateralization, such as in Blois-Heulin *et al.* (2012), Leliveld *et al.* (2010), Palmer (2002).

Calculations were performed in the R-project for statistical computing (www.r-project.org, version 2.13.1). The Bonferroni correction procedure was applied to the p-value taking into account the fact that the measurements of various parameters were performed on the same sample (Glantz 1994).

RESULTS

The CC of adult *G. locorojo* were found to be elongate bodies closely adjoined to the aorta. The CC ventral bridge between their caudal parts is well discernible. The CA are separately located ellipsoid bodies joined to the CC and suboesophageal ganglion via corresponding (allatal) nerves. Thus, the complex consisting of the CC, the CA, and the allatal nerves surrounds the sagittally located pharynx (Figure 2), which is typical for gryllids (Cazal 1948; Gaude 1975).

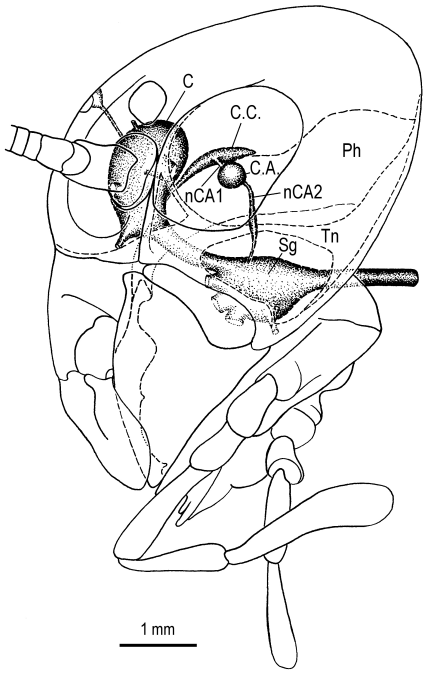


Figure 2. The RCC location inside the female head, lateral view (two figures were made before and after preparation and combined into one figure; fresh material prepared in 0.75 % NaCl). Additional designations: nCA1, *nervus corporis allati* I; nCA2, *nervus corporis allati* II; Sg, suboesophageal ganglion; Tn, tentorium.

The maximal head width expressed as Me (q1; q3) of adult females was 6050 μm (5925 μm; 6138 μm), the relative width of RCC (in % of the maximal head width) was 18.8 % (18.7 %; 19.1 %). The data confirming the rightward lateralized location of CC within the RCC and of RCC elements within the head capsule are presented in Figure 3. The left *C/Wmax*, *A/Wmax*, and *R/Wmax* were 53.60 % (53.16 %; 53.72 %), 41.53 % (41.34 %; 42.38 %), and 5.883 % (5.750 %; 6.233 %), respectively. The right *C/Wmax*, *A/Wmax*, and *R/Wmax* were 46.40 % (46.28 %; 46.84 %), 39.60 % (39.03 %; 40.33 %), and 1.633 % (1.566 %; 1.706 %), respectively. In each of ten females measured, the left *C/Wmax* and *R/Wmax* were larger than their right counterparts, and the true probabilities of rightward lateralization of the CC bridge inside the head capsule and in the whole RCC were not equal to 0.5 (p=0.002 and 0.006, for *C/Wmax* and *R/Wmax*, respectively). The left *A/Wmax* was larger than right *A/Wmax* in 8 of 10 females measured (in 2 females, the left and right *A/Wmax* were equal to each other), and the true probability of rightward lateralization of whole RCC inside the head capsule was not equal to 0.5 (p=0.008).

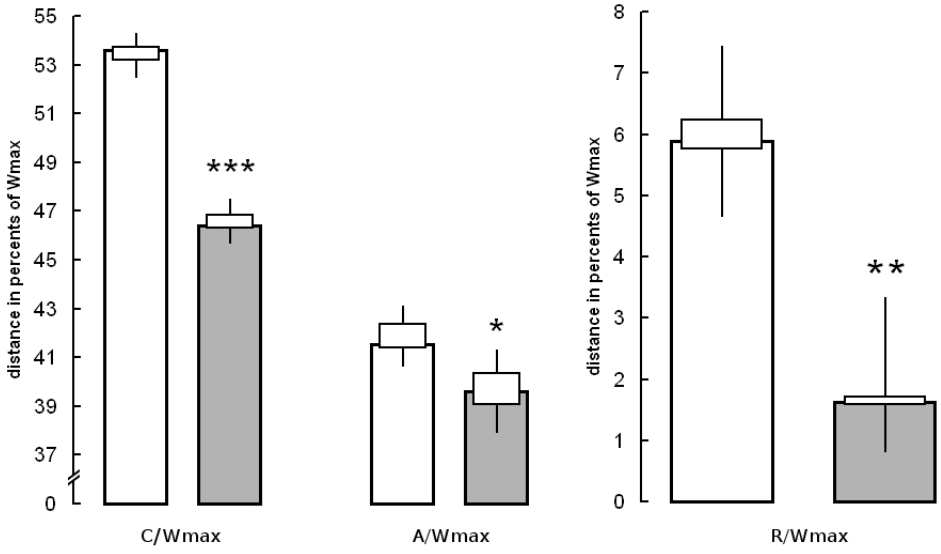


Figure 3. Plots of RCC topography parameters of adult female crickets, *Gryllus locorojo*, showing the asymmetry biases. **Note:** The columns represent the median values, the ends of boxes represent the first and third quartiles, the ends of whiskers represent the minimum and maximum values; the left distance median values are designated by white columns, the right distance median values are designated by gray columns. C/Wmax,

the distances from the left and right temples to the projection of the caudal (ventral) CC bridge on the line connecting the temples related to the maximal head width; *A/Wmax*, the distances from the left and right temples to the projection of most lateral contour of nearest *corpus allatus* on the line connecting the temples related to the maximal head width; *R/Wmax*, the distances from the projections of the medial borders of left and right CA to the projection of the CC bridge caudal point on the line connecting the temples related to the maximal head width. *****, ******, and ******* designate the significant differences as compared to corresponding left distances (p-values are, 0.002, 0.006, and 0.008, respectively; the sign z-test with the application of the Bonferroni correction procedure to the p-value). In 2 of 10 females, the left *A/Wmax* was equal to the right *A/Wmax*.

Fresh RCC extirpated in 0.75 % NaCl, retains its asymmetric shape after careful dissection. The right-sided bias of CC location inside the head capsule can also be observed through the occipital opening without the preliminary removal of the epicranium. It seems to be a common feature of morphology of both females and males (Figure 4).

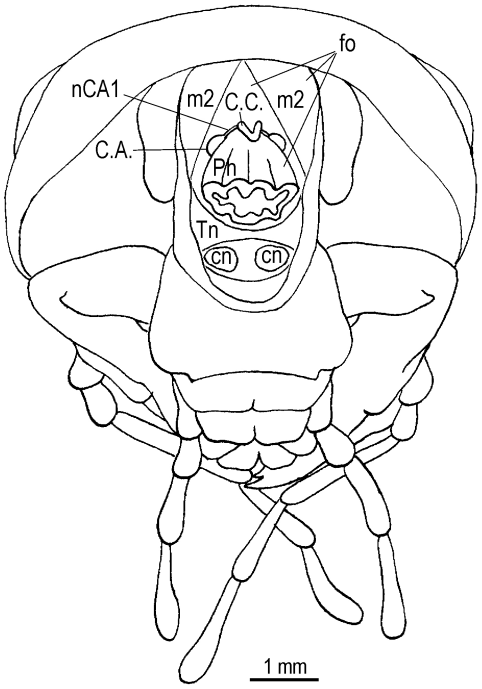


Figure 4. Asymmetric location of CC in the RCC inside the head capsule of a male *G. locorojo*, caudal view through the occipital opening (fresh material prepared in 0.75 % NaCl). Additional designations: fo, foramen occipitale (occipital opening); m2, mandibular adductor muscle; cn, suboesophageal-prothoracic connective.

DISCUSSION

The most prominent (rightward) asymmetry in the location of RCC parts was found in case of the CC bridge (as it follows from the comparisons of left *C/Wmax* and *R/Wmax* with their right counterparts) while the whole RCC (as expressed by left and right *A/Wmax*) has almost central position in the head capsule. The most probable explanation of the CC right-sided bias is the right-sided location of the aorta, the head vessel that tightly connects with the medial surfaces of CC. The slightly asymmetric location of CA revealed in most cases can be due to their junction with CC via short *nervi corporis allati* I. Since the CC location asymmetry is notable within the unprepared head capsule (Figure 4), the observed feature cannot be explained by preparation carelessness. Also, it needs to be underlined that the CC rightward lateralization is discernible in fresh material (not treated with ethanol), and the ethanol-treated crickets were used just for confirmation of this observation.

Probably, this is the first report of RCC lateralization in crickets. However, considering published literature, it can be suggested that this asymmetry in the relative position of RCC parts is not a unique feature of *G. locorojo*. The original illustrations in some papers include the figures of distinctly asymmetric RCC (with the biased CC) of house crickets, *Acheta domestica* (Belyaeva 1964; Neuhäuser *et al.* 1994; Stay *et al.* 1994; Thomsen 1943) and field crickets, *Gryllus campestris* (Cazal 1948) and *Gryllus bimaculatus*, (Neuhäuser *et al.* 1994) without mentioning the RCC asymmetry in the descriptions. In cases of *Acheta domestica* in Belyaeva (1964) and *Gryllus bimaculatus* in Neuhäuser *et al.* (1994), the CC are biased to the right. The other papers contain figures of RCC slices or figures of RCC where it is

not clear whether RCC are oriented dorsal-up or ventral-up, therefore, the direction of CC lateralization (rightward or leftward) cannot be distinguished.

No such asymmetry seems to be present in *Teleogryllus commodus*, judging from figures in Moore & Loher (1988) and Pipa & Moore (1988), but these figures are schematic. If the RCC of *Teleogryllus commodus* is really symmetric, in context of the phylogenetic relations described for Gryllidae in Weissman *et al.* (2012), the rightward lateralization of CC will be considered as symplesiomorphy or homoplasy in species of genera *Acheta* and *Gryllus*.

Also, judging from figures in Cazal (1948), the structural asymmetry of RCC can be supposed to be a feature of some species of insect taxa, other than Orthoptera. Right side lateralization of fused CA seems to be present in *Stenopsocus immaculatus* (Psocoptera). Antisymmetrically located CA (not fused with each other) may also be found in insects, such as *Clonopsis gallica* (Phasmida): in this species, according to figure in the same source, the left *corpus allatus* is located more frontally than the right *corpus allatus*. Considering this, it is easy to expect the further findings of the various RCC asymmetries and antisymmetries in insect taxa which are not analysed yet.

It is difficult to explain the functional importance of CC and whole RCC asymmetries found in *G. locorojo*. Apparently, there are no data concerning the differences between the left and right parts of CC related to their neurohemal function, but the specific unequal distribution of stored neurohormones among the left and right lobes of CC can be a reason of CC bridge anatomical lateralization. It should also be taken into consideration that the RCC connects with other structures, and some of them, such as the aorta, are also asymmetrically located inside the head capsule, as it was mentioned above. The rightward lateralization of more caudal parts of the aorta seems to be very probable, and it can be tested in the future studies. It is not excluded that the location of the CC-aorta complex in crickets may be influenced by anatomical traits of other organs adjoined to it, such as pharynx or salivary glands, and such asymmetries of CC and aorta location might have no functional relevance.

Detailed measurements of CC and CA location may also be performed on the adult male crickets of this species. The RCC glands location in the crickets of pre-adult stages are also of great interest. To test the hypothesis that the RCC rightward asymmetry is a common feature of gryllid anatomy, measurements of topography of CC and CA in other cricket species (primarily of genera *Gryllus* and *Acheta*) should also be performed. In conclusion, in the adult cricket, *Gryllus locorojo*, the CC location within the head capsule and within the RCC is prominently asymmetric, right-side biased. The whole RCC is also right-side biased within the head capsule in most cases. The right side lateralization of CC bridge and whole RCC is confirmed statistically in females (p less than 0.01 in all tests).

ACKNOWLEDGEMENTS

This paper has been formed on the basis of my unpublished thesis defended on the Department of Entomology of the St. Petersburg State University in 2001. So I would like to thank the advisor, Dr A.N. Knyazev, for his kind assistance, and Dr A.G. Akimov for introduction to handling the crickets. I'm grateful to reviewers of this paper for their very useful comments including important suggestions which were helpful for me to consider the topic much more deeply. I would also like to thank them and G.O. Kerkeshko and A.V. Khalin for their help in finding the articles cited.

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Recibido abril 16, 2013, publicado diciembre, 2013